HUNTINGTON MEDICAL RESEARCH INSTITUTES
NEUROLOGICAL RESEARCH LABORATORY
734 Fairmount Avenue
Pasadena, California 91105

Contract No. NO-NS-82388

QUARTERLY PROGRESS REPORT

January 1 - March 31, 1999

Report No. 5

"SAFE AND EFFECTIVE STIMULATION OF NEURAL TISSUE"

William F. Agnew, Ph.D.
Ted G.H. Yuen, Ph.D.
Douglas B. McCreery, Ph.D.
Leo Bullara, B.A.

ABSTRACT

During the past quarter, we evaluated the histologic effects of varying the speed at which 19-microelectrode arrays are inserted into the brain. Six animals (9 electrode sites) were implanted with the unpulsed multi-electrode arrays into either the sensorimotor cortex or parietal cortex or both. Both acute (4-5 hour) or chronic (15-28 day) experiments were conducted. Our objective was to develop a method by which the spatially dense arrays could be inserted while inflicting minimal damage to the brain's microvasculature. Insertion speeds of 1 m/sec appeared to be optimum from the perspective of minimizing trauma to tissues surrounding the microelectrodes.

INTRODUCTION

The overall goals of this study are to develop safe and effective methods for microstimulation of the cerebral cortex preliminary to the implementation of visual and auditory prostheses. In this period, we have begun the histologic evaluation of electrode sites in the postcruciate and parietal cortex of the cat using a new 19-microelectrode array (Fig. 1). The electrodes were implanted using a microelectrode inserted operated at various insertion speeds. None of these arrays was pulsed in order to determine the trauma induced by large numbers of microelectrodes and the histologic effect of various insertion speeds.

METHODS

Fabrication of recording microelectrode arrays. The shafts of the discrete iridium microelectrodes are made from iridium wire, 35 μm in diameter, which has been etched down from larger diameter wire. One end of each shaft is etched electrolytically to a cone with an included angle of 10° and with a blunt tip approximately 12 μm in diameter.

After the tips have been etched to the proper shape, a Teflon-insulated wire lead is micro-welded near the upper end of the shaft. The shaft and weld junction is then insulated with 4 thin coats of Epoxylite electrode varnish, and each layer of insulation is

baked using a schedule recommended by the manufacturer. When properly cured, Epoxylite insulation remains intact in vivo for at least 12 months, and some electrodes have been functioning *in vivo* for 2 to 5 years (unpublished results).

The insulation is removed from the tip of the shaft by an erbium laser, to yield an active geometric surface area of 2,000 +/- 300 μm^2. Next, the individual microelectrodes are assembled into arrays, using a Teflon mold, 3 mm in diameter and 0.5 mm deep. In the bottom of the mold cavity are holes for 19 separate alignment tubes, spaced 380 µm apart (center-to-center). The 16 active microelectrodes are inserted into the tubes, to a depth of 1.1 or 1.2 mm, and 3 electrically inactive, uninsulated stabilizing shafts, each 75 µm in diameter, are inserted into separate tubes, to a depth of 2.5 mm. The upper portion of the electrodes, including the lead wire weld junction are encapsulated in medical grade 2-part epoxy (Masterbond EP21LY), to form the array superstructure.

After extrusion from the mold, the top of the array superstructure is held against a vacuum wand, and with the aid of a special microscope eyepiece, the individual electrodes are aligned to the axis of the wand, so that they will not slash the cortex when they are inserted into the brain. A complete array is depicted in Figure 1. The array is then cleaned, and gas-sterilized.

Surgical Implantation of Intracortical Electrodes. Adult cats of either sex were anesthetized with nitrous oxide and Halothane. The aseptic surgical procedure is carried out with the animal's head in a stereotaxic apparatus. The scalp and temporalis muscle were reflected and, using a Hall bone drill, a craniectomy was made over the left frontal cortex extending into the frontal sinus, and over the parietal cortex. Using the bone drill, the bone margin on the caudal edge of the craniectomy was contoured into a ramp to accommodate the array cable. The frontal sinus was partly filled with bone cement.

A patch of dura, somewhat larger than the array's superstructure matrix, was resected from the postcruciate or parietal cortex, and the array of microelectrodes was inserted into the cortex using the inserter tool, which is mounted on the stereotaxic

frame. The tool originally was developed to facilitate implantation of microelectrode arrays into the human cochlear nucleus.

Prior to deployment, the microelectrode array is enclosed and protected within the end of the barrel (stator), and is held against the end of the sliding armature by a vacuum to keep it aligned with the axis of the tool. The distal end of the stator is slotted, to accommodate the cable. When the trigger is released, the array, still held by the vacuum against the end of the armature and aligned with the axis of the stator, is pushed into the tissue at a moderate velocity (0.75 to 4 m/sec). The array is then covered with a sheet of fascia resected from the temporalis muscles. The wound is then closed in layers. and the cat given appropriate post-surgical care.

Histologic Methods. At the termination of the implant tissues, the cats were anesthetized with Nembutal and perfused through the ascending aorta with ½-strength Karnovsky's fixative. The array of 19 microelectrodes was removed from the postcruciate and/or parietal cortex after resection of the overlying tissue. The block containing the array tracks was resected, embedded in paraffin, sectioned serially in the horizontal plane (perpendicular to the shafts of the iridium microelectrodes) at a thickness of 8 μm, and stained with Nissl and H&E using alternating sections.

RESULTS

Insertion Procedures. In cat IC-177, the orifice of the introducer instrument's fixed piece (stator) was positioned over the parietal cortex so that it was just in contact with the pia, but it was not allowed to depress the surface of the brain. The introducer's sliding piece (armature) was adjusted so that at the end of the insertion stroke, it would be positioned flush with the end of the stator, causing the array matrix to be expelled completely from the end of the stator. The array was then inserted into the brain at a rather low velocity (~ 1 m/sec, as determined by subsequent frame-by-frame-analysis of the video tape). The dense electrode cluster caused the pia to dimple during the insertion process, and the electrodes did not insert fully. The insertion velocity was then increased, to approximately 4 m/sec. With this modification, the electrodes were

inserted completely into the parietal cortex. The array was then inserted into the postcruciate gyrus, using the same settings. The cat was sacrificed 15 days after implanting the array, and the histologic analysis showed severe and widespread injury to the neurons and neuropil subjacent to the array, with evidence of interstitial hemorrhages and gliosis (Fig. 2). We therefore conducted a series of acute experiments, in which the cats were sacrificed 4 to 5 hours after implanting the arrays (Table 1).

In cat IC-179, the armature was adjusted so that the array matrix was not completely extruded from the stator. This was done to avoid having the underside of the matrix slap against the surface of the brain at the end of the insertion stroke. The insertion velocity was quite low (~ 0.75 m/sec). These settings inflicted very little tissue injury and few if any microhemorrhages near the electrode tracks, but the electrodes did not insert fully.

In cat IC-180, we made 2 modifications to our insertion procedure. Prior to inserting the array, and after contacting the pia, the stator was advanced by 1 mm, so that the surface of the brain was "pre-dimpled". Our expectation was that the brain would be less inclined to be dimpled further as the electrodes were penetrating the pia, and the array would be inserted fully. We also increased the insertion speed, to approximately 4.5 m/sec. The array inserted fully, but there were microhemorrhages near some of the electrode tracks.

In cats IC-182 and IC-184, we implanted the arrays at a lower velocity (~1 m/sec) after pre-dimpling the cortex by 1 mm. In cat IC-182, there were few microhemorrhages, but there was widespread edema in the vicinity of the array. A frame-by-frame analysis of the video tape revealed that the bulky cable had displaced the array laterally after the inserter tool had been withdrawn. Therefore, in cat IC-184, the same insertion procedure was used (the electrode was inserted at approximately 1 m/sec, after pre-dimpling the pia) and the cable was sutured to the dura after the array had been inserted, but while it was still stabilized by the armature of the inserter tool. The histologic evaluation of this animal revealed a low incidence of microhemorrhages, all

TABLE I

IMPLANTATION OF 19-PIN ARRAYS

CAT #	IMPLANT PERIOD	LOCATION	INSERTION SPEED(*)	PRE-DIMPLING(§)	HISTOLOGY SUMMARY					
					HEMORR.	CAVITAT.	SCAR	EDEMA	GLIOSIS	NEURONS
177	**15 days**	**Post-cruciate**	**High (~4m/sec)**	**None**	**1 el. & all stab.**	**10 tracks & all stab.**	**0**	**Most tracks**	**All tracks**	**"Smudgy" (damaged), entire array site**
179	4 hrs	Post-cruciate	Low (~0.75 m/sec)	None	0	0	0	0	0	Stellate at all stab.
180	4.5 hrs	Post-cruciate	High (~4m/sec)	1 mm	2 el. All stab.	0	0	All stab.	0	Flat at all stab
180	4 hrs	Parietal	High (~4m.sec)	1 mm	2 el. All stab.	0	0	All stab. at low level	0	Flat at some el & all stab
182	4.5 hrs	Post-cruciate	Low (~1m/sec)	1 mm	0	0	0	Entire array site	0	Occas. flat at el & all stab.
184	5 hrs	Post-cruciate(+)	Low (~1 m/sec)	1 mm	4 el. All stab.	0	0	2 el. & all stab. at lower levels	0	Flat &/or damaged at 2 el. & 2 stab.
184	5 hrs	Parietal(+)	Low (~1 m/sec)	1 mm	0	0	0	Lat. & ant. stab.	A few glia at med. stab.	Occas. flat at all stab
186	**28 days**	**Post-cruciate(+)**	**Low (~1 m/sec)**	**1 mm**	**El. #7 at pia.**	**Most el. (surface only)**	**5 el. at 100 µm depth**	**0**	**El. & all stab. (All levels)**	**Occas. flat at all stab.**
186	**28 days**	**Parietal(+)**	**Low (~1 m/sec)**	**1 mm**	**0**	**0**	**0**	**0**	**0**	**Occas. flat at all stab.**

(*) The final insertion was at least 2 mm from the sites of the previous insertions
(§) the cortex is depressed by the orifice of the tool, prior to inserting the array
(+) The cable was sutured to the dura before retracting the insertion tool
Bold indicates a chronic implant.
El. = Electrode track
Stab. = Stabilizer track

small and confined to the lumen of the electrode tracks or their immediate vicinity (Fig. 3). The same procedure was then used to implant the two arrays chronically (for 28 days) into the parietal and cruciate cortex of cat IC-186. One mild hemorrhage was observed at one of 16 sites in the postcruciate; however, scarring was seen at 5 of 16 sites, indicating a previously resolved hemorrhage (100 μm below the pial surface). No hemorrhage was seen in the site in the parietal cortex nor other evidence of neural damage other than occasional flattening of neurons near the stabilizing electrode tracks.

Summary.

Hemorrhage. The sites of 4 of the 6 array injected at a low speed, showed no hemorrhage and one additional site showed only a small amount of blood confined to the lumen of one of the 19 tracks (Table 1). Conversely, the 3 array sites where the higher insertion speed was used showed hemorrhage associated with some electrodes and all stabilizer tracks.

Cavitation. "Cavitation" is defined as vacuoles in the tissue surrounding the tracks, and the presence of residual hemosiderophages. Cavitations were present at two of the three chronically implanted array sites. Animal IC-186 (postcruciate gyrus) showed cavitations close to the electrode tracks only at the pia. In this animal, erythrocytes were confined to the track of electrode #7.

Scars. Of the two animals having chronic (15 to 28 days) implants (using low insertion speed), only one array site contained gliotic scars near the electrode tracks. This was the array in the gyrus suprasylvius of Animal IC-186 (28-day implant period). The scars were well developed and circumscribed 5 electrode tracks down to a depth of 100 μm.

Edema. Of the 9 implant sites, 6 exhibited various degrees of edema, sometimes involving all stabilizer track sites, and a few or all of the electrode sites. The latter occurred in IC-182 despite the slower insertion speed. The remaining 6 arrays showed edema near some of the electrode tracks and near the stabilizing pins, irrespective of the insertion speed (Fig. 4).

Gliosis. Aside from a single stabilizer track site which was accompanied by a few glial cells, all acute, low and high speed insertion sites were free of glial cell accumulations. At all chronic implant

sites, most or all tracks, including stabilizer tracks, were accompanied by small accumulations of glial cells (Fig. 5).

DISCUSSION

Correlation of insertion speed with the histological analysis of the implant sites showed an association between the presence of hemorrhage and insertion speed (Table 1). Four of the 6 array sites, at which a slow insertion speed was used, showed no hemorrhage and one additional array site showed only a few erythrocytes in a single electrode track but no extension to surrounding tissue. The remaining slow-speed insertion sites showed minimal hemorrhage at several electrode sites and all stabilizer tracks. On the other hand, all array sites subjected to fast insertion of the electrodes showed hemorrhages near the electrode tracks.

These findings will be studied further in future implant series although, at the present, it appears that the faster insertion speed does not permit adequate time for the blunt-tipped microelectrodes to push the blood vessels aside. Further, it may be that, in the rare cases of hemorrhage following slow insertion, some blood vessels were directly in the path of the electrode tip and puncture or tearing were inevitable.

Cavitations, as described in previous series, indicate a previous hemorrhagic event in which many, if not all, extravasated erythrocytes have been scavenged and usually, residual hemosiderophages are present. The eventual resolution of these sites will take the form of a compact glial or connective tissue scar. The fact that the scars are present at only one or a few levels indicates that the cause is due to disruption of blood vessel(s) at that level and not to misalignment of the electrode. Otherwise, the misaligned shaft would have disrupted and torn the tissue for the entire length of the shaft, and a scar would be present at every level.

The localized edema observed in the acute experiment (Fig. 4) may be inevitable with the insertion of large numbers of electrodes at either speed of insertion. The lack of edema around the electrodes of the 28-day implant suggests that the edema is transitory. Although glial cell accumulation at sites of tissue damage reveals the exquisite sensitivity of their response, at only a single track was there an accumulation of glia within the 4 to 5 hour acute implants. These cells were a common finding at track sites in the more chronic (15 to 28 day) implants. The flat neurons' presence near electrode and stabilizer tracks in this series has been seen in many previous experiments in this laboratory. The etiology appears to be compressive forces exerted on the

neuropil adjacent to the newly created track. The narrow, distorted profiles of such neurons persisted as long as one year following electrode implantation, indicating that the mechanical plasticity of the cortex is not as great as previously suspected. The finding of stellate neurons near all of the stabilizer tracks in IC-179 is unexplained but may suggest that the sheer size of the stabilizer pins will, in some instances, exert a somewhat damaging mechanical force even at the lower (1 m/sec) rate of insertion.

The poorly defined, damaged neurons, coupled with hemorrhages and cavitations throughout the entire array site in IC-177 are probably the result of the array matrix impacting on the brain cortex. These sequelae remained even 15 days after implantation.

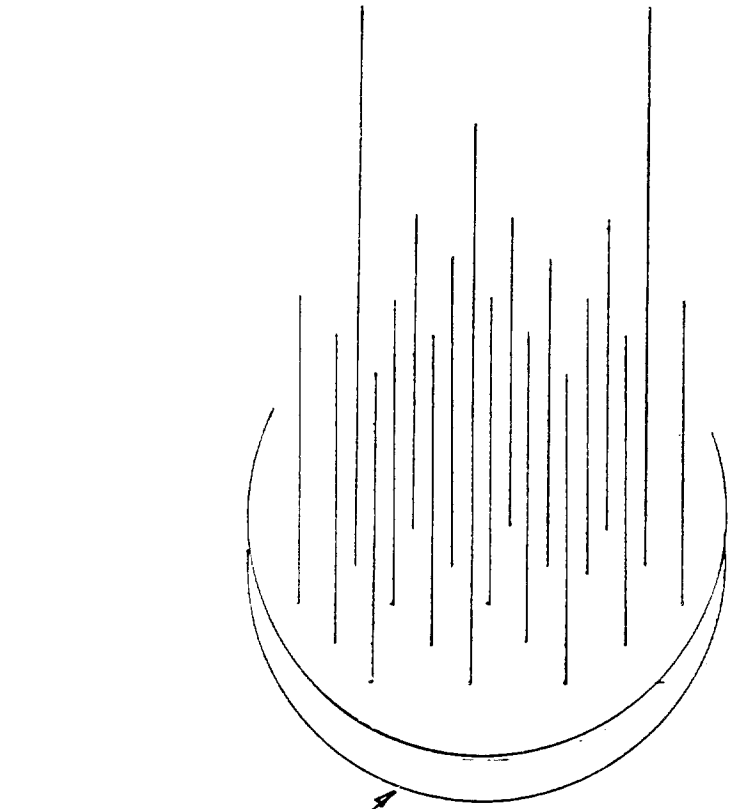
CONCLUSIONS

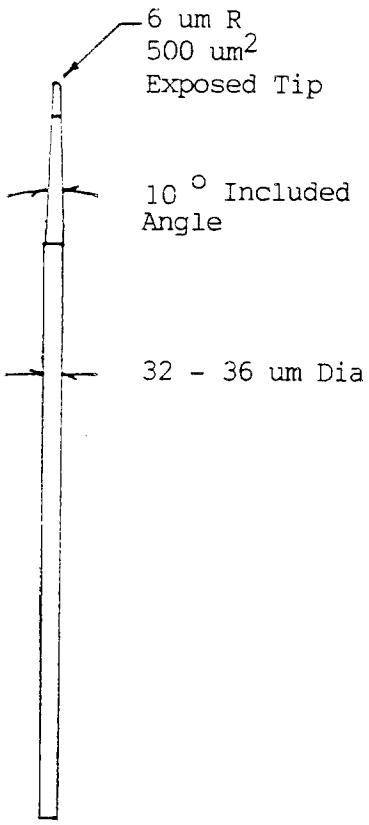
Studies to date in the sensorimotor and parietal cortex, indicate that insertion speeds of 1 m/sec appear to be optimum from the perspective of minimizing trauma. Insertion speeds slower than 1 m/sec appear to be impractical when inserting arrays of many (e.g., 16) closely-spaced microelectrodes in that incomplete insertion and increased dimpling result. Predimpling of the brain by the orifice of the insertion tool is a helpful procedure in that it minimizes displacement of the brain during the insertion process and thereby allows the use of a lower insertion velocity.

PENTAGONAL IRIDIUM ELECTRODE ARRAY

Specifications:

Electrode Shaft Material:	Pure Iridium
Shaft Size:	32 - 36 μm dia 1.5 - 3.0 mm lengths
Shaft Insulation:	Epoxylite
Electrode Activated Surface:	500 μm² Iridium Oxide
Number of Electrodes:	19
Electrode Distribution:	Center Electrode: 1.5 mm long Inner Circle: 1.4 mm lengths Outer Circle: 1.5 mm lengths Anchor Electrodes on Outer Circle: 3.0 mm long
Electrode Spacing:	380 μm center to center





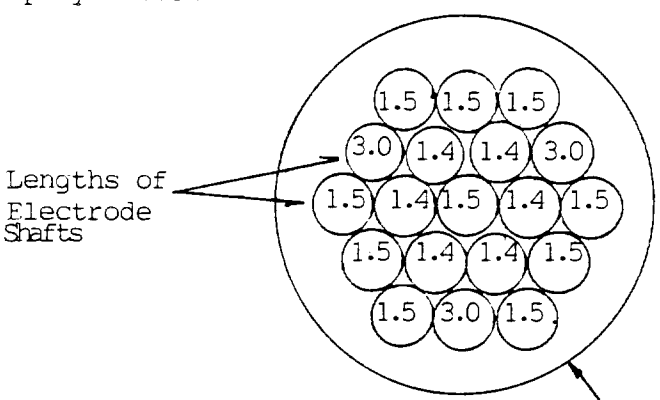


Fig. 1

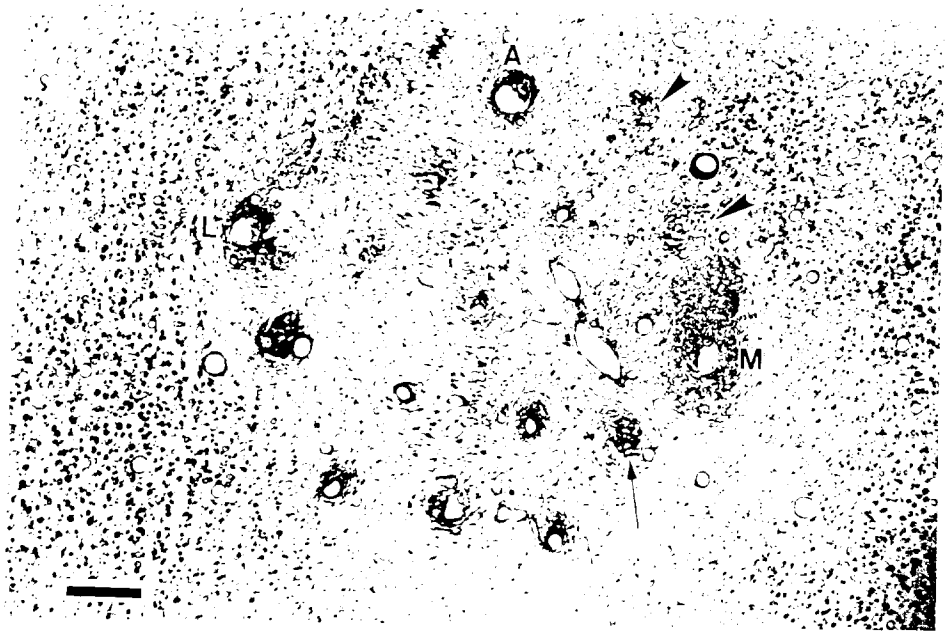


Fig. 2. IC-177. Postcruciate gyrus. High speed insertion. Implant duration 15 days. Depth = 600 µm. Slight to moderate gliosis is present at all track sites, including the stabilizer tracks. L = lateral stabilizer track, A = anterior stabilizer track, M = medial stabilizer track. At high magnification, the neurons under the entire array appeared ill defined ("smudgy") as compared to neurons immediately outside the array site. The sectioning has reached the tip of electrode no. 8 (arrow). The tips of electrode nos. 15 and 16 have been passed although gliosis below their tips is present (arrowheads). Nissl stain. Bar = 250 µm. This and all succeeding micrographs are from paraffin-embedded horizontal sections.

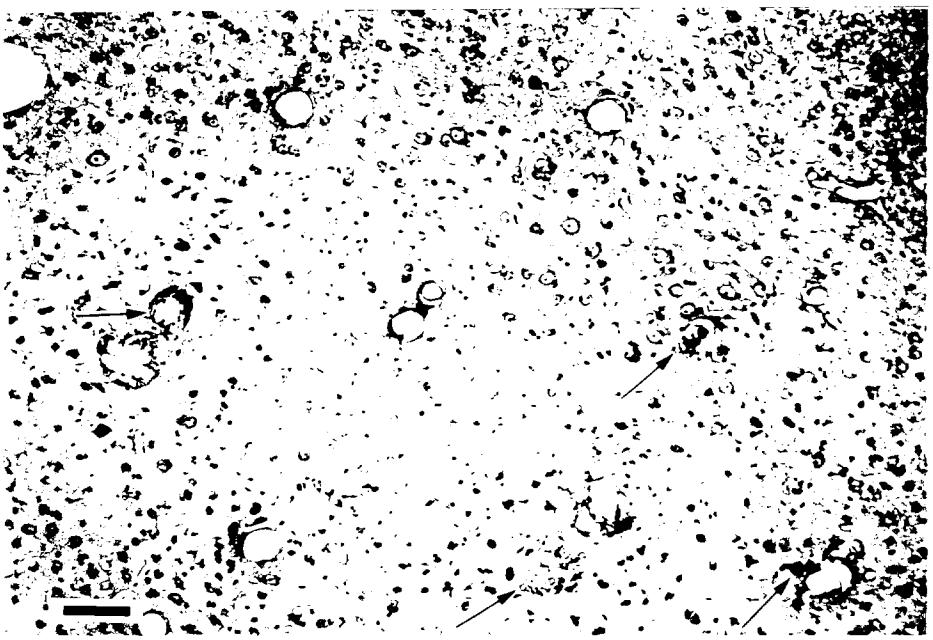


Fig. 3. IC-184. Postcruciate gyrus. Low speed insertion. Implant duration = 5 hrs. Depth = 200 µm. Four of the 16 electrode tracks are accompanied by slight hemorrhages (arrows). The area in the center of the micrograph shows the pallor of moderate edema. A few neurons near the left of the micrograph show perineuronal haloes. H&E stain. Bar = 100 µm.

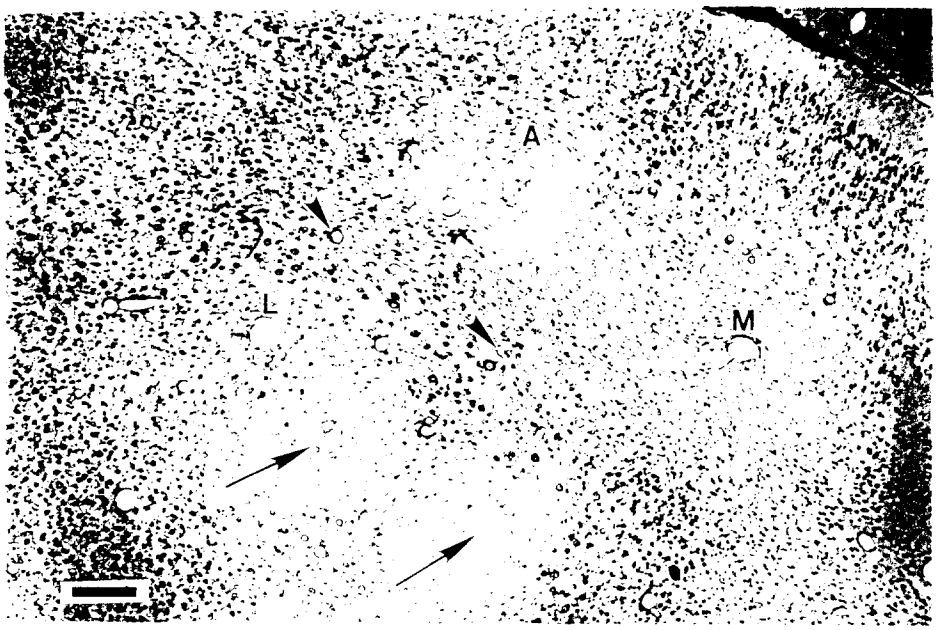


Fig. 4A. Same array site as that shown in Fig. 3 but slightly deeper (220 µm). Four prominent areas of edema are evident. These surround the anterior and medial stabilizer tracks (A and M, respectively) and electrode track nos. 10 and 12 (arrows). Two other electrode tracks show less extensive edema around them (arrowheads). Nissl stain. Bar = 250 µm.

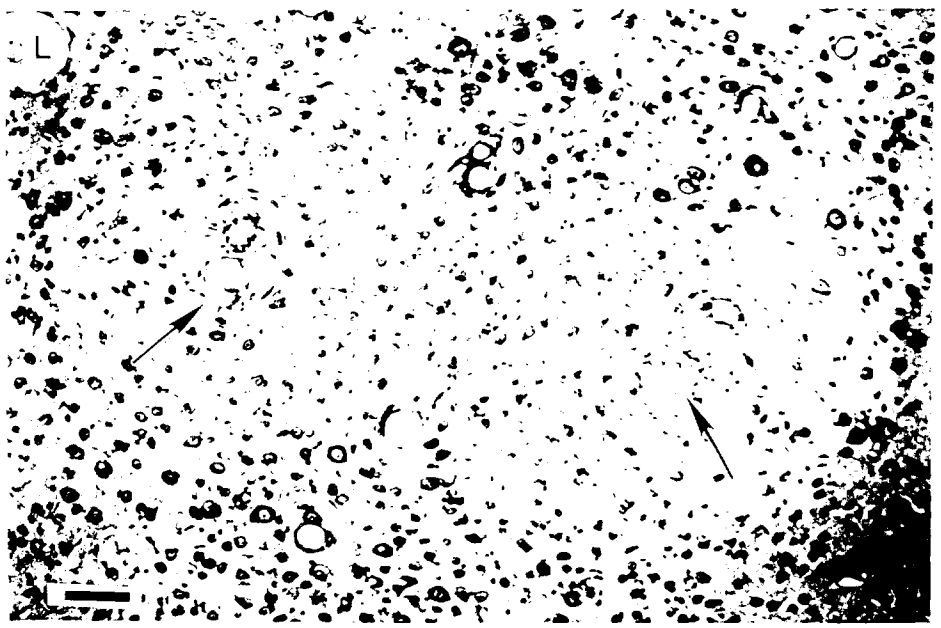


Fig. 4B. Higher magnification of a portion of the previous micrograph. Moderate edema surrounds electrode nos. 10 and 12 (arrows). Note the circular areas of pallor surrounding each track. L = lateral stabilizer track. Nissl stain. Bar = 100 µm.

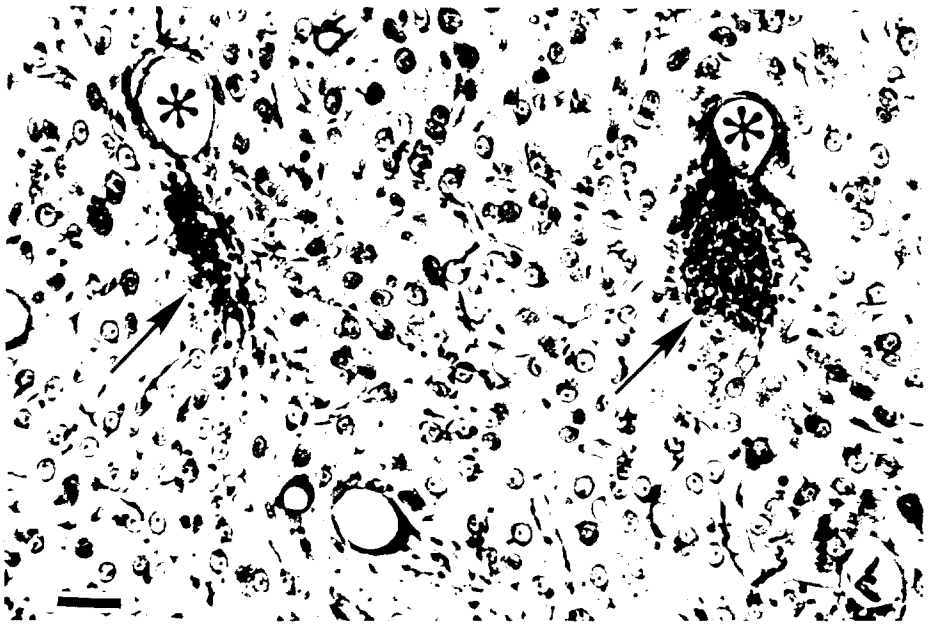


Fig. 5. IC-186. Left postcruciate gyrus. Low speed insertion. Implant duration = 28 days. Depth = 200 µm. Two of 5 electrode tracks (asterisks) which were accompanied by gliosis (arrows) are shown. All nearby neurons appear normal. Nissl stain. Bar = 50 µm